Supporting Information

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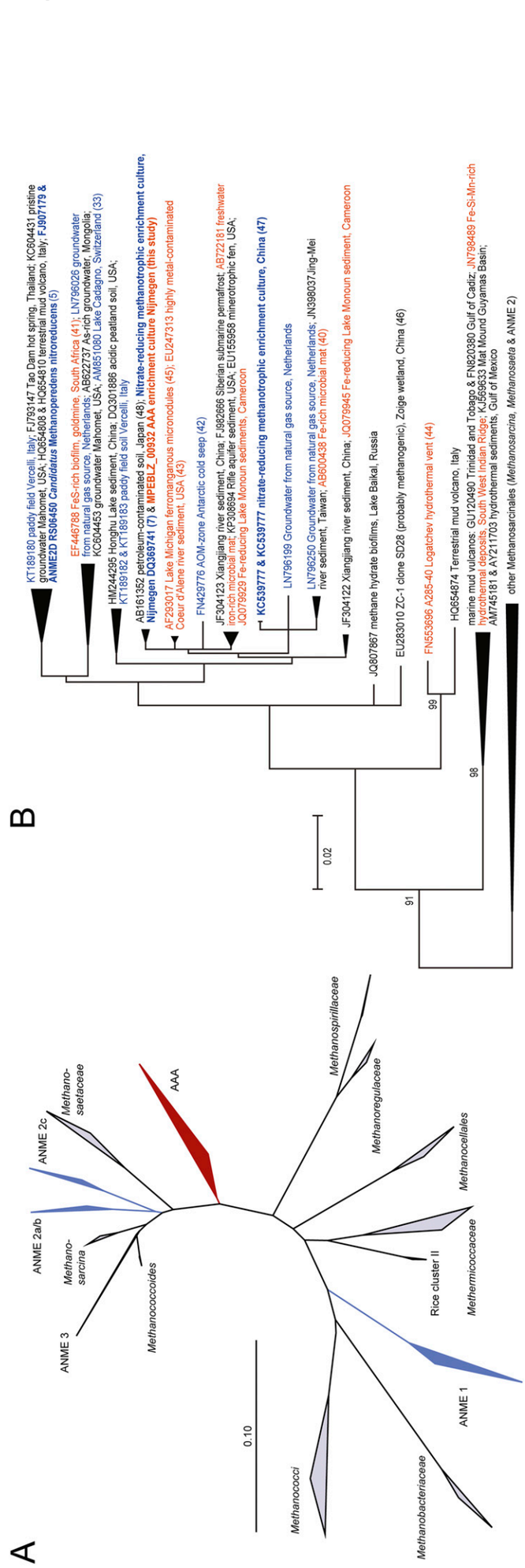

Fig. S1. (*A*) Phylogenetic position of AOM-associated archaea (AAA) (red) among other archaeal methanotrophs (blue) and methanogens (gray) based on sequences of 16S rRNA gene sequences obtained from GenBank. (*B*) Detailed phylogenetic tree of 16S rRNA genes of the AAA group within *Methanosarcinales*. Sequences from enrichment cultures are highlighted in bold letters; sequences from sites or cultures with demonstrated AOM activity are shown in blue; those from particularly metal-rich or iron-reducing environments are shown in orange. The evolutionary analysis was conducted with the maximum-likelihood algorithm implemented in MEGA6 (39). There were a total of 949 positions in the final dataset. Bootstrap support >90 is shown at the branches. (Scale bar, number of substitutions per site.)

Table S1. Multi(>2)-heme *c*-type cytochromes predicted from the contigs assigned to AAA in the metagenome of the enrichment culture

Locus tag	Protein length, no. of amino acids	CxxCH and other motifs	No. of transmembrane helices	Best hit identifier	Best hit organism	Best hit identity
MPEBLZ_00008	358	4	0	gi|851163073|ref|WP_048092705.1|	*Candidatus* Methanoperedens nitroreducens	44.5
MPEBLZ_00010	190	6	1	gi|851163076|ref|WP_048092707.1|	*Ca.* M. nitroreducens	60.5
MPEBLZ_00012	319	4	1	gi|851163096|ref|WP_048092715.1|	*Ca.* M. nitroreducens	61.2
MPEBLZ_00015	253	7	1	gi|851163104|ref|WP_048092719.1|	*Ca.* M. nitroreducens	60.8
MPEBLZ_00016	343	4	0	gi|851163073|ref|WP_048092705.1|	*Ca.* M. nitroreducens	45.3
MPEBLZ_00238	317	11	2	gi|630830727|gb|KCZ72437.1|	*Ca.* M. nitroreducens	60.6
MPEBLZ_00452	577	9	0	gi|851163118|ref|WP_048092727.1|	*Ca.* M. nitroreducens	50.4
MPEBLZ_00526	864	16	1	gi|851162463|ref|WP_048092373.1|	*Ca.* M. nitroreducens	37.9
MPEBLZ_00803	1,846	7*	1	gi|851163104|ref|WP_048092719.1|	*Ca.* M. nitroreducens	54.5
MPEBLZ_00816	316	6†	0	gi|851164534|ref|WP_048093459.1|	*Ca.* M. nitroreducens	71.9
MPEBLZ_00817	309	6†	2	gi|851164537|ref|WP_048093461.1|	*Ca.* M. nitroreducens	63.2
MPEBLZ_01070	678	9‡	0	gi|851160641|ref|WP_048091456.1|	*Ca.* M. nitroreducens	60.6
MPEBLZ_01114	437	5*	1	gi|851165424|ref|WP_048093932.1|	*Ca.* M. nitroreducens	74.5
MPEBLZ_01115	167	4	0	gi|630828680|gb|KCZ70396.1|	*Ca.* M. nitroreducens	61.1
MPEBLZ_01126	288	2	2	gi|490648195|ref|WP_004513190.1|	*Geobacter metallireducens*	43.4
MPEBLZ_01329	202	5	1	gi|851154723|ref|WP_048088734.1|	*Ca.* M. nitroreducens	57.4
MPEBLZ_01361	1,137	16	1	gi|851162463|ref|WP_048092373.1|	*Ca.* M. nitroreducens	40.3
MPEBLZ_01741	696	21	0	gi|851163401|ref|WP_048092876.1|	*Geoglobus acetivorans*	28.6
MPEBLZ_01742	561	11	0	gi|851155354|ref|WP_048089050.1|	*Ca.* M. nitroreducens	31.9
MPEBLZ_01743	914	18	1	gi|851149563|ref|WP_048086421.1|	*Ferroglobus placidus*	30.0
MPEBLZ_01744	606	6†,‡	1	gi|851163118|ref|WP_048092727.1|	*Ca.* M. nitroreducens	59.3
MPEBLZ_01877	828	16‡	1	gi|851163401|ref|WP_048092876.1|	*G. acetivorans*	38.2
MPEBLZ_01950	432	8	0	gi|851165403|ref|WP_048093919.1|	*Ca.* M. nitroreducens	69.1
MPEBLZ_02010	573	6	0	gi|851163118|ref|WP_048092727.1|	*Ca.* M. nitroreducens	51.1
MPEBLZ_02042	403	5	2	gi|851156284|ref|WP_048089356.1|	*Ca.* M. nitroreducens	67.5
MPEBLZ_02500	373	7	1	gi|851153793|ref|WP_048088239.1|	*Ca.* M. nitroreducens	74.2
MPEBLZ_02501	179	5	0	gi|851153793|ref|WP_048088239.1|	*Ca.* M. nitroreducens	67.1
MPEBLZ_02503	469	8	0	gi|851153795|ref|WP_048088240.1|	*Ca.* M. nitroreducens	69.1
MPEBLZ_02608	209	5	0	gi|851160641|ref|WP_048091456.1|	*Ca.* M. nitroreducens	51.5
MPEBLZ_02874	305	7‡	2	gi|630831567|gb|KCZ73276.1|	*Ca.* M. nitroreducens	41.3
MPEBLZ_02879	267	7	2	gi|851162380|ref|WP_048092326.1|	*Ca.* M. nitroreducens	54.6
MPEBLZ_03194	318	6†	1	gi|630831820|gb|KCZ73529.1|	*Ca.* M. nitroreducens	71.2
MPEBLZ_03195	149	4‡	1	gi|630831821|gb|KCZ73530.1|	*Ca.* M. nitroreducens	70.5
MPEBLZ_03279	655	2	1	gi|635652775|ref|WP_024299646.1|	*Methylosarcina lacus*	68.3
MPEBLZ_03539	237	5‡	1	gi|630831567|gb|KCZ73276.1|	*Ca.* M. nitroreducens	78.1
MPEBLZ_04272	521	12*	1	gi|851149560|ref|WP_048086419.1|	*F. placidus*	27.5
MPEBLZ_04274	227	4	1	gi|851166986|ref|WP_048094540.1|	*Geoglobus ahangari*	35.6
MPEBLZ_04276	224	5	1	gi|851163417|ref|WP_048092885.1|	*G. acetivorans*	27.7
MPEBLZ_04299	324	6†	2	gi|851155273|ref|WP_048089031.1|	*Ca.* M. nitroreducens	61.9
MPEBLZ_04300	364	13†	1	gi|851155276|ref|WP_048089032.1|	*Ca.* M. nitroreducens	73.0
MPEBLZ_04301	246	2	1	gi|258591543|emb|CBE67844.1|	*Ca. Methylomirabilis oxyfera*	26.2

Prediction of multiheme cytochrome *c* proteins was done using a perl script (prot_motif_search.pl) available at https://github.com/dspeth followed by BLAST analysis of conservation of the heme-binding motifs in related sequences. Prediction of transmembrane helices was performed using TMHMM (www.cbs.dtu.dk/services/TMHMM/) (49). Each superscript denotes one divergent heme binding motif.

*CxxCK.

†CxxxCH.

‡CxxxxCH.